Kick it up a Notch: NOTCH1 activation in T-ALL

While the human *NOTCH1* gene initially was cloned as part of a translocation breakpoint in T cell acute lymphoblastic leukemia (T-ALL) tumors, this translocation is present in only a small percentage of T-ALL patients. A recent paper by Weng et al. (2004) demonstrates that novel types of activating mutations in the *NOTCH1* gene occur in more than half of all T-ALL cases, implicating *NOTCH1* as a major player in the etiology of T-ALL.

The Notch signaling pathway is a conserved intercellular signaling mechanism, and mutations in Notch pathway components disrupt embryonic development in diverse organisms and cause inherited disease syndromes in humans (reviewed in Artavanis-Tsakonas et al., 1999; Gridley, 2003). Genes of the Notch family (*NOTCH1-NOTCH4* in mammals) encode single-pass transmembrane receptors that bind to transmembrane ligands encoded by genes of the Delta and Jagged/Serrate families. NOTCH family proteins are proteolytically processed both during transit to the cell surface and upon ligand binding. In mammals, NOTCH precursors (for example, NOTCH1) in the secretory pathway are cleaved in the extracellular domain by a furin-type protease. This results in formation of a NOTCH1 heterodimer consisting of noncovalently associated extracellular and transmembrane subunits. The extracellular subunit contains the signal peptide and 36 tandemly repeated copies of an epidermal growth factor-like motif. The transmembrane subunit contains a short extracellular domain, the membrane-spanning region, and an intracellular domain (termed ICN1) containing several conserved motifs. Stable association of the two NOTCH1 subunits is dependent on a newly described heterodimerization domain comprising the carboxy-terminal end of the extracellular subunit and the extracellular amino-terminal end of the transmembrane subunit (Sanchez-Irizarry et al., 2004). Upon interaction with a ligand-expressing cell, additional proteolytic cleavages occur that result in receptor activation and signal transduction. The final cleavage, which occurs within the plasma membrane and releases ICN1 into the cytosol, is catalyzed by the γ-secretase complex. Once released, ICN1 translocates to the nucleus, where it participates in the formation of a large transcriptional complex that activates NOTCH target gene transcription.

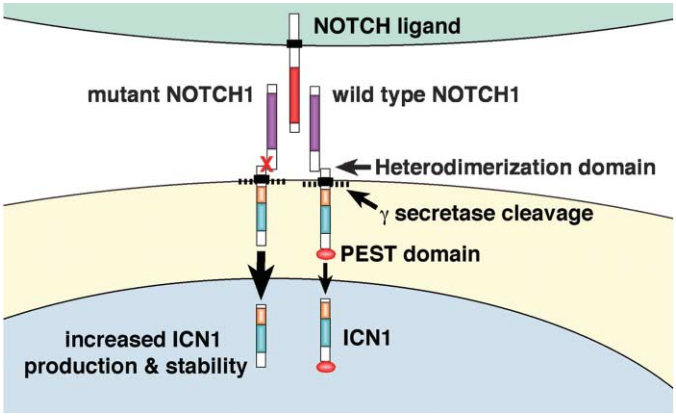

Figure 1. Model for NOTCH1 activation by heterodimerization domain and PEST domain mutations

In the wild-type NOTCH1 protein (right), stable association of the furin-cleaved extracellular and transmembrane subunits requires the heterodimerization domain. Upon interaction with a NOTCH ligand-expressing cell, two additional proteolytic cleavages occur in the NOTCH1 protein. The final cleavage, catalyzed by γ-secretase, releases the NOTCH1 intracellular domain (ICN1), which can then translocate to the nucleus. In the mutant NOTCH1 protein (left), Weng et al. (2004) propose that heterodimerization domain missense mutations (red X) increase levels of ICN1 by enhancing γ-secretase cleavage, while the PEST domain truncations increase ICN1 half-life.

It has been known for more than a decade that disregulated expression of the intracellular domains of Notch family receptors contributes to the formation of several cancers in mammals (reviewed in Radtke and Raj, 2003; Zweidler-McKay and Pear, 2004). The human NOTCH1 gene, the first mammalian Notch homolog isolated, was identified through the cloning of a t(7;9) chromosomal translocation found in a subset of human T-ALL (Ellisen et al., 1991). This translocation brings the disrupted *NOTCH1* gene under the transcriptional control of the T cell receptor β locus and results in the disregulated expression of intracellular forms of the NOTCH1 protein that resemble ICN1. Work in mouse models revealed that ICN1 expression in bone marrow resulted in T cell leukemia in 100% of the transplanted mice (Zweidler-McKay and Pear, 2004). However, despite the efficacy of ICN1 expression in inducing T cell leukemia in mouse models, it was puzzling that the t(7;9) translocation was present in less than 1% of human T-ALL tumors.

A recent paper by Weng and colleagues now provides an explanation for this enigma (Weng et al., 2004). These authors had shown previously that sustained NOTCH1 signaling was required for proliferation and survival of a T-ALL cell line containing the t(7;9) translocation. In the new study, Weng et al. assessed whether growth of other T-ALL cell lines lacking the t(7;9) translocation was sensitive to growth arrest induced by inhibition of γ-secretase activity. They found that several T-ALL cell lines exhibited G_0/G_1 cell cycle arrest when cultured in the presence of a γ-secretase inhibitor. They then sequenced the NOTCH1 gene in these cell lines and found mutations in sequences encoding two different domains of the NOTCH1 protein in four of the five cell lines that exhibited γ-secretase-dependent proliferation. They observed missense mutations of conserved amino acid residues in the heterodimerization domain of the NOTCH1 protein and frameshift mutations that led to truncation of the PEST domain (a motif implicated in the regulation of protein turnover) at the carboxy terminus of the NOTCH1 protein. Importantly, both

types of mutation were present in *cis* in the same *NOTCH1* allele in all four cell lines.

Weng et al. then examined primary T-ALL tumors for the presence of NOTCH1 mutations and frequently found mutations in the same two domains. At least one mutation was identified in more than 50% of T-ALL bone marrow samples, which included representatives of all major molecular subtypes of T-ALL (Ferrando et al. 2002). To prove that these mutations activated NOTCH1 function, Weng et al. performed transcriptional reporter assays with NOTCH-sensitive reporters. They found activation of transcriptional activity in both single heterodimerization domain mutations (3- to 9-fold increase in activity) and PEST domain mutations (1.5- to 2-fold increase). Interestingly, combining these two types of mutation in *cis* in the same *NOTCH1* allele led to striking synergy of transcriptional activation, resulting in a 20- to 40-fold increase in activity. Transcriptional stimulatory effects of these mutant NOTCH1 proteins were completely abrogated by culturing transfected cells with a γ-secretase inhibitor.

These mutations represent a novel means to activate Notch signaling function. While the mechanistic details are currently unknown, Weng et al. propose a model for the synergy of the two types of mutations (Figure 1). They suggest that the heterodimerization domain mutations increase the rate of production of ICN1 by enhancing γ-secretase cleavage, while the PEST domain truncations increase ICN1 half-life. These predictions remain to be verified experimentally. These results help explain the contradiction between the efficacy of T cell leukemia induction by activating NOTCH1 function in mouse models and the rare occurrence of the t(7;9) translocation in human T-ALL tumors. The t(7;9) translocation can occur only in the small subset of committed T cell progenitors undergoing V-D-J recombination at the T cell receptor β locus, while the NOTCH1 missense and frameshift mutations could occur at many additional stages of T cell development, including the hematopoietic stem cell or the common lymphoid progenitor.

These new findings also have therapeutic implications. Under currently utilized therapeutic regimens, the 5 year survival rate for pediatric and adolescent T-ALL patients is approximately 60%–75% (Ferrando et al., 2002). Since both transcriptional activation and cell proliferation caused by the NOTCH1 heterodimerization and PEST domain mutations are dependent on γ-secretase-mediated proteolytic cleavage, γ-secretase is a potential therapeutic target in cases of T-ALL refractory to current therapeutic approaches. Small molecule γ-secretase inhibitors are being intensively studied as therapeutics for Alzheimer's disease (reviewed in Wolfe, 2002). However, potential side effects of γ-secretase inhibitors, due to their inhibition of Notch signaling activity, remain a concern. A recent study showed that administration of a γ-secretase inhibitor to mice had deleterious effects on the lymphoid and intestinal systems (Wong et al., 2004). Furthermore, mice with a reduction in gene dosage of the two presenilin genes (*Psen1*$^{+/-}$ *Psen2*$^{-/-}$ double mutant mice), which encode components of the γ-secretase complex, develop an age-dependent myeloproliferative disease (Qyang et al., 2004). These results suggest that caution may be required when introducing γ-secretase inhibitors into the clinical arena. Nevertheless, the findings of Weng et al. transform the role of NOTCH1 activation in the etiology of T-ALL from bit player to star and raise hopes for novel therapeutic approaches to this disease.

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Selected reading

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